Diagnostic Screening Method for the Determination of Trichothecene Exposure in Animals

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A rapid screening method for diagnosis of exposure to the trichothecene mycotoxins T-2, diacetoxyscirpenol (DAS), deoxynivalenol (DON), and nivalenol (NIV) has been developed. The toxins are extracted from urine and plasma with a C-18 cartridge followed by a Florisil minicolumn cleanup. The residues are then hydrolyzed with NaOH to form the parent alcohols of any esterified toxins, with a final cleanup using a silica cartridge. Quantitation of the trifluoroacyl derivatives of the parent alcohols T-2 tetraol (TOL), scirpentriol (STR), DON, and NIV is by GLC/ECD with a detection limit of better than 25 ng/mL. Mass spectral confirmation by negative chemical ionization is possible with little or no sample workup.

Trichothecene mycotoxins are sesquiterpenoids produced by several species of the fungi *Fusarium*, which often grow on agricultural products. T-2, diacetoxyscirpenol (DAS), deoxynivalenol (DON), and nivalenol (NIV) are some of the primary trichothecenes produced by *Fusaria* (Bamburg et al., 1971; Ghosal et al., 1978; Mirocha et al., 1976; Pathre et al., 1977; Smalley et al., 1973). Ingestion of trichothecene-contaminated feed has been associated with a number of adverse health affects in domestic livestock. Exposure in the bovine and porcine may result in emesis, diarrhea, lethargy, hemorrhage, reduced weight gain, decreased immune response, and/or death (Chu, 1977; Cote et al., 1984; Forsyth et al., 1977; Gilgan et al., 1966; Pathre et al., 1979; Sato et al., 1977).

T-2, DAS, and DON are extensively metabolized by a variety of animal species. Many metabolites of T-2 and DAS have been detected in the blood, urine, feces, bile, and tissues of exposed animals including swine (Bauer et al., 1985; Corley et al., 1985), poultry (Chi et al., 1978; Visconti et al., 1985; Yoshizawa et al., 1980a, 1982), rats (Matsumoto et al., 1978; Ohta et al., 1978; Sakamoto et al., 1986; Ueno, 1977), and cattle (Pawlosky et al., 1984; Yoshizawa et al., 1981). It has been recently reported that several deepoxide metabolites of T-2, DAS, and DON have been found in rats, pigs, and cattle (Chatterjee et al., 1986; Corley et al., 1986; Cote et al., 1986a,b; Sakamoto et al., 1986; Yoshizawa et al., 1980b, 1983, 1985, 1986). The structures of T-2, DAS, DON, and NIV along with their various metabolites are shown in Figures 1 and 2.

Trichothecene metabolites exhibit a wide range of chemical behavior due to the varied number and types of side groups. Current methods for analysis of T-2, DAS, DON, or NIV along with their metabolites in biological samples can be lengthy or difficult. Standards of many of the metabolites are not commercially available and may be costly or difficult to synthesize or maintain. The method presented in this paper utilizes four commercially available standards (T-2 tetraol, scirpentriol, deoxynivalenol, nivalenol) and is a rapid and easy method to screen for T-2, DAS, DON, or NIV exposure in animals using urine or plasma. Deepoxytrichothecene metabolites can be detected; however, the availability of deepoxy standards is very limited at the present time.

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EXPERIMENTAL SECTION

Materials and Reagents. Vacuum manifold box (Vac Elut) and Bond-Elut C-18 cartridges with 500 mg or 1 g of packing were purchased from Analytichem International (Harbor City, CA). Prep-Sep silica cartridges with 300 mg of packing were purchased from Fisher Scientific (Itasca, IL). Five-milliliter polypropylene minicolumns with filter paper disks and bottom closures were purchased from IsoLabs, Inc. (Akron, OH).

Solvents were glass-distilled acetone, acetonitrile, chloroform, ethyl acetate, hexane, methanol, and toluene from EM Science (Cherry Hill, NJ) and HPLC-grade glacial acetic acid from J. T. Baker Chemical Co. (Phillipsburg, NJ). Trifluoroacetic anhydride (TFAA) and pentafluoropropionic anhydride (PFPA) were purchased from Pierce Chemical Co. (Rockford, IL). TFAA and PFPA are corrosive and react violently with water. 4-(Dimethylamino)pyridine (DMAP) was purchased from Sigma Chemical Co. (St. Louis, MO).

Nivalenol was purchased from Wako Chemical USA, Inc. (Dallas, TX). Deoxynivalenol and diacetoxyscirpenol were purchased from Myco Labs (Chesterfield, MO). T-2 was obtained from fungal cultures of *Fusarium sporotrichioides* grown in our laboratory. T-2 tetraol and scirpentriol were prepared by the alkaline hydrolysis of T-2 and diacetoxyscirpenol, respectively (Wei et al., 1971). Deepoxy compounds were prepared by incubating T-2, diacetoxyscirpenol, or deoxynivalenol with rumen microorganisms under an anaerobic environment, to produce deepoxy metabolites (Swanson et al., 1987). The deepoxy metabolites of T-2 and diacetoxyscirpenol were converted by alkaline hydrolysis to form deepoxytetraol and deepoxyscirpentriol, respectively. Trichothecenes are cytotoxic and potent protein synthesis inhibitors and should be handled with care.

Gas chromatograph: Hewlett-Packard Model 5840A equipped with an ^{63}Ni electron capture detector and 7672A autosampler.

Columns and conditions: for the packed column, 6 ft × 2 mm (i.d.) glass column packed with 3% OV-17 on 100/120 Supelcoport from Supelco Inc. (Supelco Park, PA); gases, argon–methane (95:5) carrier at 35 mL/min; injector temperature, 275 °C; detector temperature, 300 °C; oven temperature, 165 °C (145 °C for analysis of the deepoxide metabolites, also); for the large-bore capillary column, Megabore DB-1701, 15 m × 0.527 mm (i.d.), 1-μm film thickness from J&W Scientific (Folsom, CA); gases, He carrier at a linear velocity of 55 cm/s and argon–methane (95:5) makeup for a total flow of 55 mL/min;

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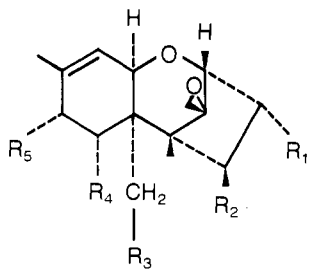
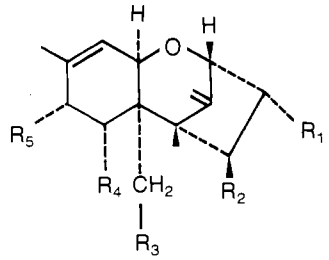


	R1	R2	R3	R4	R5
T-2	OH	OAc	OAc	H	X1
HT-2	OH	OH	OAc	H	X1
T-2 triol (TRI)	OH	OH	OH	H	X1
T-2 tetraol (TOL)*	OH	OH	OH	H	OH
neosolaniol (NEO)	OH	OAc	OAc	H	OH
4-deacetylneosolaniol (4DN)	OH	OH	OAc	H	OH
3′-OH-T-2	OH	OAc	OAc	H	X2
3′-OH-HT-2	OH	OH	OAc	H	X2
3′-OH-triol	OH	OH	OH	H	X2
4-acetyltetraol	OH	OAc	OH	H	OH
8-acetyltetraol	OH	OH	OH	H	OAc
diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
scirpentriol (STR)*	OH	OH	OH	H	H
deoxynivalenol (DON)*	OH	H	OH	OH	O
nivalenol (NIV)*	OH	OH	OH	OH	O
fusarenon-X (FUSX)	OH	OAc	OH	OH	O
3-acetyldeoxynivalenol	OAc	H	OH	OH	O
15-acetyldeoxynivalenol	OH	H	OAc	OH	O

Figure 1. Structures of various trichothecenes. Key: X1, $(CH_3)_2CHCH_2C(O)O$; X2, $(CH_3)_2C(OH)CH_2C(O)O$;*, parent alcohol.



	R1	R2	R3	R4	R5
deepoxy-HT-2 (de-HT-2)	OH	OH	OAc	H	X1
deepoxytriol (de-TRI)	OH	OH	OH	H	X1
deepoxytetraol (de TOL)*	OH	OH	OH	H	OH
deepoxy-3′-OH-HT-2	OH	OH	OAc	H	X2
deepoxymonoacetoxyscirpenol (de-MAS)	OH	OH	OAc	H	H
deepoxyscirpentriol (de-STR)*	OH	OH	OH	H	H
deepoxydeoxynivalenol (DOM-1)*	OH	H	OH	OH	O

Figure 2. Structures of various deepoxide trichothecenes. See Figure 1 for key to abbreviations.

injector temperature, 275 °C; detector temperature, 300 °C; oven temperature, 190 °C (180 °C for analysis of deepoxide metabolites, also).

Gas chromatograph/mass spectrometer: extranuclear (Extrel) Simulscan 300 series quadrupole with a Perkin-Elmer Sigma 2 gas chromatograph.

Column and conditions: DB-1701, 30 m × 0.25 mm (i.d.), 0.25-μm film thickness from J&W Scientific (Folsom, CA); He carrier gas, linear velocity 50 cm/s; injection mode, splitless with a 30-s purge time, or split with a ratio of 1:10; injector temperature, 270 °C; splitless temperature program, 100–225 °C at 20 °C/min, hold at 225 °C for 5 min; split temperature program, 250–275 °C at 5 °C/min, hold at 275 °C for 5 min.

Mass spectrometer: negative chemical ionization with methane; electron energy, 300 eV; interface temperature, 270 °C; source temperature, 100 °C. Trifluoroacyl derivatives of the toxins were formed. Mass of the M + H ion: nivalenol, 697; T-2 tetraol, 683; scirpentriol, 571; DON, 585; deepoxytetraol, 667; deepoxyscirpentriol, 555; deepoxy-DON, 569.

Methods. *C-18 Extraction.* With a vacuum manifold box, a 500-mg C-18 cartridge was preconditioned with 3 mL of methanol followed by an equal volume of water and the solvents were discarded. The cartridge was not allowed to go to dryness. Centrifuged urine (1 mL) or diluted plasma (2 mL) (diluted 1:1 with water) was added to the cartridge. The sample was slowly loaded over approximately 30 s by applying a gentle vacuum. One milliliter of water was added to the cartridge, the vacuum applied for 30 s, and then the solvent discarded. Methanol (2 mL) was added to the cartridge, the vacuum applied, and the eluate collected in a 3-mL test tube. The methanol eluate was concentrated to 0.1–0.2 mL under a gentle stream of nitrogen in a 50 °C water bath, but not to complete dryness. Any residue adhering to the sides of the test tube was redissolved by vigorous mixing.

Florisil Minicolumn Cleanup. A 0.65-g quantity of Florisil (60–100 mesh) was wet-packed in ethyl acetate into a capped minicolumn. Approximately 5 mm of sodium sulfate was layered above and below the packing. Ethyl acetate (0.8 mL) was added to the C-18 methanol eluate and the resultant solution mixed and transferred to the column. The test tube was rinsed with 2 × 1 mL of ethyl acetate, and the contents were transferred to the column. The column was uncapped and the eluate collected in a 8-mL screw-cap test tube. To the column was added 4 mL of ethyl acetate when the previous 3-mL portion reached the top of the column packing. The eluate was collected in the same 8-mL test tube for a total volume of 7 mL and concentrated to dryness.

Hydrolysis. NaOH (0.1 N, 0.1 mL) in 95% methanol was added to the residue, and the resultant mixture was capped and mixed to dissolve any residue adhering to the sides of the test tube. It was left at room temperature (~23 °C) for 40 min. Fifty microliters of 0.25 N acetic acid in toluene was added to neutralize the base and the mixture vortexed.

Silica Cartridge. With use of a vacuum manifold box, a silica cartridge was preconditioned with 3 mL of chloroform–acetone (1:3) followed by an equal volume of hexane, not allowing the cartridge to go to dryness. To the hydrolysis solution was added 3 mL of chloroform–hexane (1:4) and the resultant solution mixed. The contents were immediately transferred to the cartridge, vacuum was applied, and then the solvent was discarded. The test tube that contained the hydrolysis residue was rinsed with 1 mL of chloroform–acetone (95:5), the contents were transferred to the cartridge, vacuum was applied, and then the solvent was discarded. One milliliter of chloroform–acetone (1:3) was added to the test tube; the contents were mixed and then transferred to the cartridge. A 5-mL portion of chloroform–acetone (1:3) was added directly to the cartridge and the vacuum applied, collecting all of the chloroform–acetone (1:3) eluate in a screw-cap test tube. The contents were concentrated to dryness.

Derivatization for Gas Chromatographic Analysis. The residue was redissolved in 1 mL of toluene–acetonitrile (9:1) containing 2 mg/mL of DMAP. To this was added 50 μL of TFAA (or PFPA to form the pentafluoropropionyl derivatives when using the large-bore capillary column), and the contents were capped and then mixed. This was heated at 60 °C for 20 min cooled to room temperature, and then 1 mL of 5% sodium bicarbonate was added. On a vortexing mixer, the solution was mixed until the top

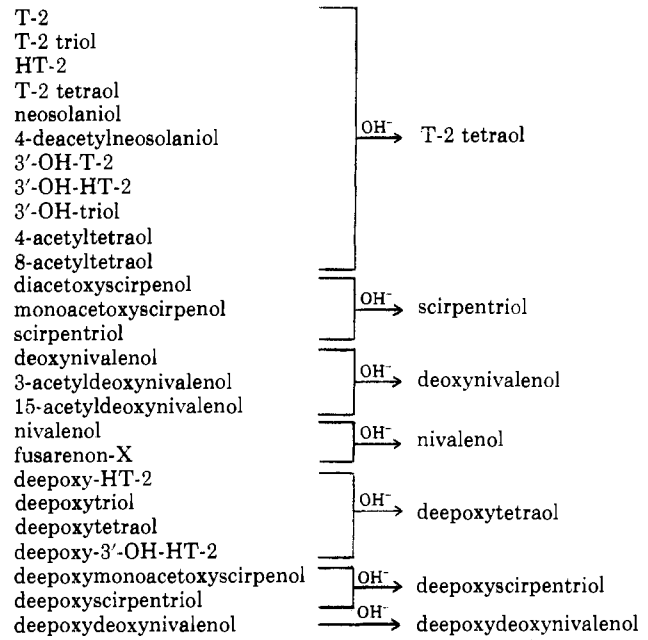


Figure 3. Identity of parent alcohols after hydrolysis with NaOH.

layer was clear and the contents were centrifuged at >6000*g* for 2–3 min. Of the top layer, 50 μL was transferred to an autosampler vial or screw-cap test tube. Hexane (0.95 mL) was added, and the contents were thoroughly mixed. Into a gas chromatograph equipped with an electron capture detector was injected 1–4 μL.

Derivatization for Mass Spectral Analysis. The same method as for gas chromatographic analysis was used; however, reduced solvent volumes may be necessary to achieve the sensitivity required for low-level (ng/mL) mass spectral analysis.

RESULTS AND DISCUSSION

The method presented in this paper is a rapid and simple screening procedure using urine or plasma for determining trichothecene exposure of animals. Only four standards (T-2 tetraol (TOL), scirpentriol (STR), deoxynivalenol (DON), nivalenol (NIV)) are necessary to screen for T-2, DAS, DON, or NIV exposure in a single analysis; the standards are readily available through several chemical supply houses. For confirmation, gas chromatography/mass spectroscopy can be performed with little or no additional workup of the samples extracted for gas chromatographic analysis. Disposable glassware is used throughout the method to eliminate the possibility of contamination or carryover from previous samples.

In this method, T-2 and its metabolites are converted to TOL, and DAS and its metabolites are converted to STR with 0.1 N NaOH; DON and NIV do not have ester side groups and are unchanged under these conditions. TOL, STR, DON, and NIV do not contain any ester groups and are referred to as the parent alcohol for each of their respective trichothecene group. Figure 3 shows the various metabolites and their corresponding parent alcohol formed after alkaline hydrolysis. The hydrolysis conditions utilized were selected to maximize the conversion of T-2 to TOL and DAS to STR, and to minimize the degradation of DON and NIV. The detection of TOL, STR, DON, or NIV in a sample would indicate trichothecene exposure.

In a previous reported method (Rood et al., 1986), hydrolysis conditions of 5-min heating at 60 °C followed by a 10-min cooling at room temperature were described. Minor deviations from time or temperature conditions, or variations in sample matrices, could lead to incomplete hydrolysis to the parent alcohols or possible degradation of some toxins. DON and NIV are suceptible to degradation under certain alkaline conditions (Young et al., 1986). The room-temperature incubation of 40 min utilized in this method achieves complete hydrolysis with minimal degradation and is much less sensitive to minor deviations in the hydrolysis conditions and composition of sample matrix.

Table I. Comparison of Levels between Urine and Plasma Samples Previously Analyzed for T-2 or DAS and Reanalyzed for TOL or STR, Respectively, by the Method Described

Urine		
time period[a]	T-2 level,[b] μg/mL	TOL level,[c] μg/mL
30	0.49	5.56
60	0.15	9.66
120	0.05	5.90
180	nd[d]	0.98
420	nd	0.52

Plasma		
time period[a]	DAS level,[e] μg/mL	STR level,[f] μg/mL
1	1.47	1.71
5	0.74	1.34
15	0.31	0.91
35	0.12	0.65
420	nd	0.23
900	nd	0.13

[a] Minutes postdosing. [b] Concentration of T-2 only. Detection limit of 0.05 μg/μL (Beasley et al., 1986). [c] Sum of all unconjugated trichothecenes detected after conversion to T-2 tetraol by alkaline hydrolysis. [d] nd denotes none detected. [e] Concentration of DAS only. Detection limit of 0.05 μg/μL (Coppock, 1984). [f] Sum of all unconjugated trichothecenes detected after conversion to scirpentriol by alkaline hydrolysis.

Since T-2, DAS, and their metabolites are converted to a corresponding hydrolyzed form (parent alcohol), a cumulative effect results. This leads to a greater sensitivity in detecting trichothecene exposure since several different metabolites are converted to a single, corresponding parent alcohol (i.e., T-2 plus metabolites → TOL; DAS plus metabolites → STR). Also, upon derivatization to their corresponding trifluoroacyl derivatives, TOL and STR have a significantly greater response factor using an electron capture detector than the esterified metabolites. The detection limit is better than 25 ng/mL for TOL, STR, DON, and NIV.

Control porcine urine and plasma chromatograms along with spiked samples are shown in Figures 4 and 5. Chromatograms of urine from a T-2 dosed cow and plasma from a DAS dosed pig are shown in Figure 6. Selected urine and plasma from animals dosed with T-2 and DAS were reanalyzed by this procedure. TOL or STR was found in samples with previously nondetected levels of T-2 or DAS, respectively (Table I).

Recoveries were checked by spiking a large volume of porcine urine or plasma at the appropriate levels with T-2, DAS, DON, and NIV dissolved in a small volume of absolute ethanol. The spike recovery analyses were performed by several individuals in our laboratory to confirm the reproducibility of the analysis. The recoveries are given in Table II. The number of deepoxy spike replications was low due to the limited availability of the deepoxy compounds. Increased recoveries of the toxins resulted when concentration of the solvent to complete dryness was avoided after the C-18 cartridge extraction, and the concentration step after alkaline hydrolysis was eliminated. Sample residues were more difficult to transfer if the

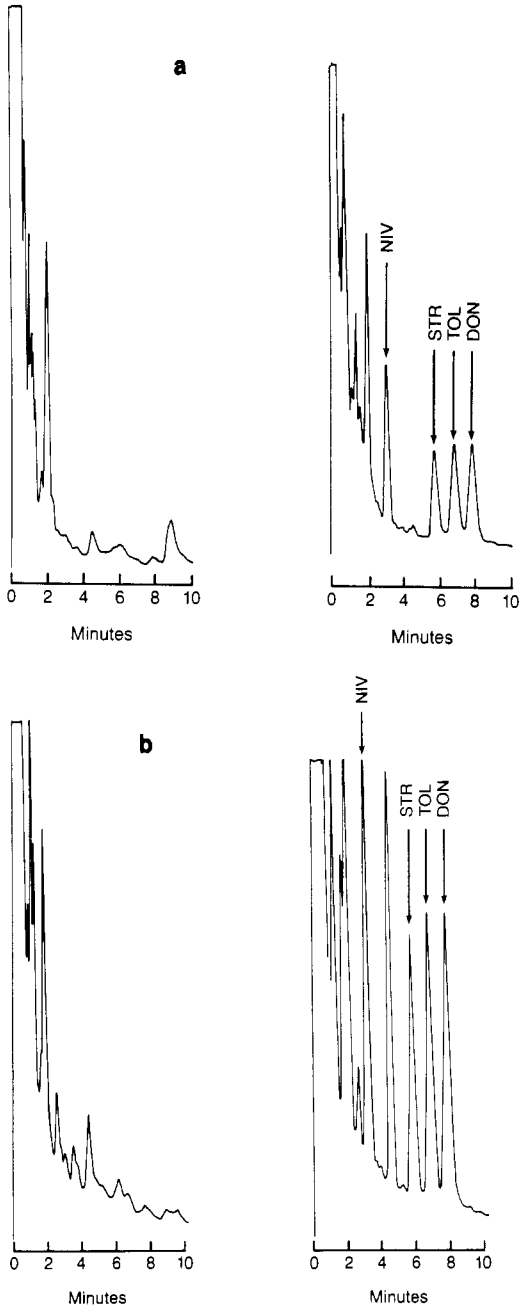


Figure 4. (a) Gas chromatograms of extracts from control pig urine (left) and pig urine spiked (right) with 125 ng/mL of NIV, DAS, T-2, and DON. Conditions: 3% OV-17 on 100/120 Supelcoport, 6 ft × 2 mm glass column; oven temperature, 160 °C. (b) Gas chromatogram of extracts from control pig plasma (left) and pig plasma spiked (right) with 250 ng/mL of NIV, DAS, T-2, and DON. Conditions: 3% OV-17 on 100/120 Supelcoport, 6 ft × 2 mm glass column; oven temperature, 160 °C.

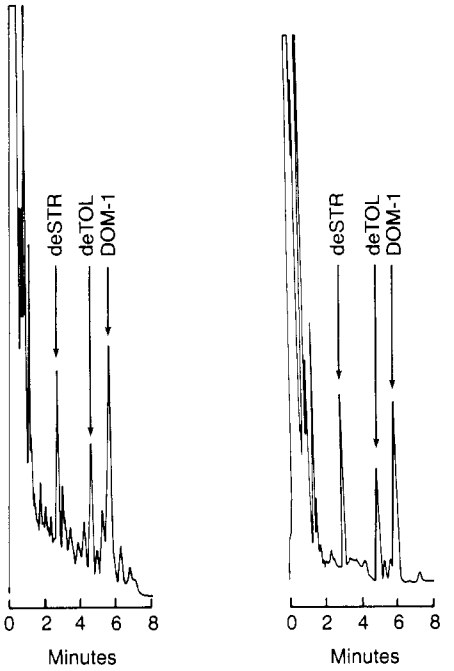


Figure 5. Gas chromatogram of extracts from pig urine (left) and plasma (right) spiked with 125 ng/mL of de-HT-2, de-MAS, and DOM-1. Conditions: 3% OV-17 on 100/120 Supelcoport, 6 ft × 2 mm glass column; oven temperature, 160 °C.

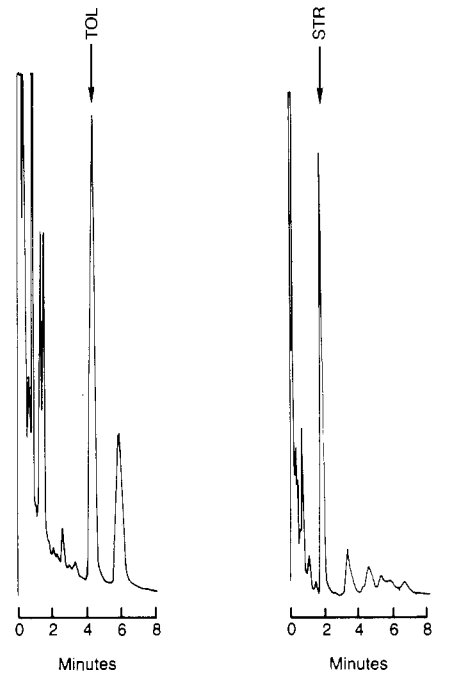


Figure 6. Gas chromatograms of extracts of urine from a T-2-dosed cow (left) and plasma from a DAS-dosed pig (right). Conditions: 3% OV-17 on 100/120 Supelcoport, 6 ft × 2 mm glass column; oven temperature, 160 °C.

solvent was completely evaporated. Eliminating or using a partial dry-down step increased the speed of the analysis, also. Improved recovery values were obtained if the silica cartridge cleanup was performed immediately after the completion of the alkaline hydrolysis step. Delays of over 1 h resulted in lower recoveries for all of the toxins.

Recently, several deepoxide metabolites of T-2, DAS, and DON have been detected as metabolites in the urine and plasma of rats, pigs, or cattle administered trichothecenes (Chatterjee et al., 1986; Corley et al., 1986; Cote et al., 1986a,b; Yoshizawa et al., 1980b, 1983, 1985, 1986). Under the hydrolysis conditions described, deepoxy metabolites of T-2 are converted to deepoxy T-2 tetraol (de-TOL), and the deepoxy metabolites of DAS are converted to deepoxyscirpentriol (de-STR); DOM-1, the deepoxy metabolite of DON, behaves in a manner similar to that of DON. Detection of a deepoxide analogue of a parent alcohol (de-TOL, de-STR, DOM-1) would indicate trichothecene exposure, also.

Analysis for the deepoxy metabolites along with their epoxide analogues can be performed with this procedure with no substantial modifications. If analysis by packed-column gas chromatography for all seven compounds is desired, a lower oven temperature is necessary to facilitate separation of the deepoxy compounds (de-TOL, de-STR, DOM-1) from the epoxide-containing compounds (TOL,

Table II. Recoveries from Plasma and Urine

	level, ng/mL	compound	recovery[a]
	a. NIV, DAS, T-2, and DON		
plasma	50	NIV	78 ± 4
		STR	89 ± 7
		TOL	76 ± 5
		DON	70 ± 2
	250	NIV	89 ± 11
		STR	85 ± 5
		TOL	66 ± 7
		DON	79 ± 4
urine	25	NIV	53 ± 10
		STR	72 ± 19
		TOL	87 ± 11
		DON	88 ± 17
	125	NIV	70 ± 5
		STR	86 ± 7
		TOL	84 ± 7
		DON	86 ± 12
	625	NIV	68 ± 4
		STR	87 ± 8
		TOL	79 ± 6
		DON	95 ± 9
	b. de-MAS, de-HT-2, and DOM-1		
plasma	125	de-STR	95 ± 4
		de-TOL	110 ± 3
		DOM-1	92 ± 3
urine	125	de-STR	94 ± 4
		de-TOL	93 ± 4
		DOM-1	82 ± 3

[a] Mean ± standard error in molar percent. n = 8 for NIV, DAS, T-2, and DON; n = 7 for de-MAS, de-HT-2, and DOM-1.

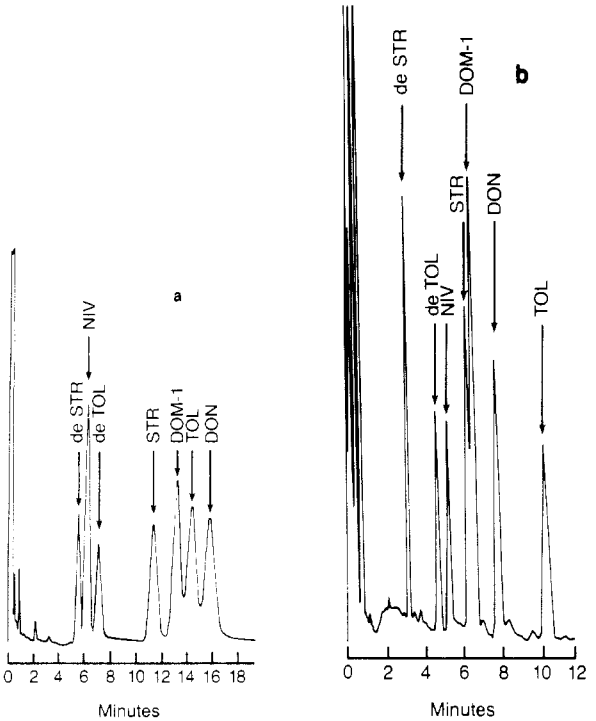


Figure 7. (a) Gas chromatogram of TFA derivatives of a standard containing parent alcohols and deepoxy parent alcohols. Conditions: 3% OV-17 on 100/120 Supelcoport, 6 ft × 2 mm glass column; oven temperature, 145 °C. (b) Gas chromatogram of PFP derivatives of a standard containing parent alcohols and deepoxy parent alcohols. Conditions: DB-1701 Megabore capillary column, 15 m × 0.527 mm; 1-μm film; oven temperature, 180 °C.

STR, DON, NIV) (Figure 7a). The recent advent of large-bore (Megabore) capillary columns has resulted in the ability to approach packed-column capacities with the separation capabilities of a capillary column. The deepoxy compounds can be separated from the epoxide compounds

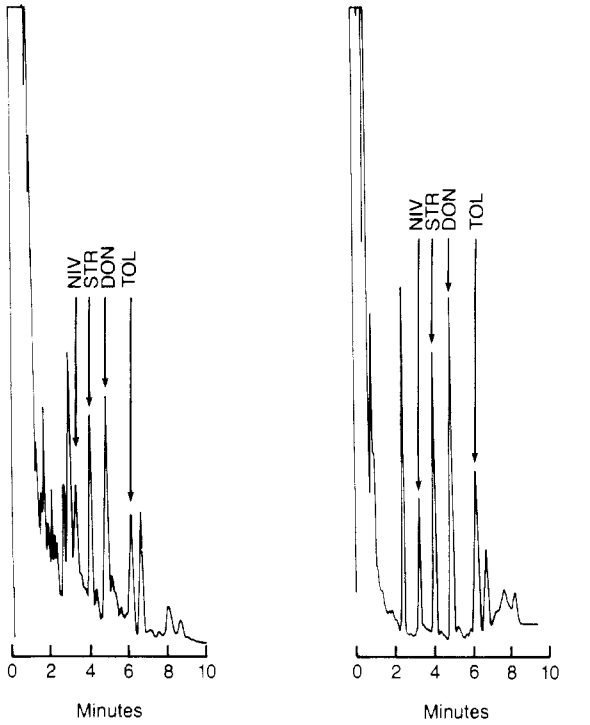


Figure 8. Gas chromatograms of extracts from pig urine spiked with 125 ng/mL (left) and pig plasma spiked with 250 ng/mL of NIV, DAS, T-2, and DON (right) (same extracts as in Figure 4, parts a and b). Conditions: PFP derivatives; DB-1701 Megabore capillary column, 15 m × 0.527 mm; 1.0-μm film; oven temperature, 190 °C.

by use of a large-bore capillary column with much shorter run times than that of a packed column (Figure 7b). One simple modification of the method was required since the TFA derivatives of NIV and STR did not separate on the DB-1701 large-bore capillary column. The pentafluoropropionyl (PFP) derivative was formed by using PFPA instead of TFAA in the derivatization procedure. The PFP derivatives are approximately 3 times more sensitive with the electron capture detector than the corresponding TFA derivatives; however, the PFP derivatives give slightly more complex chromatograms (Figure 8).

Low levels of trichothecenes detected in the urine or plasma may warrant additional confirmation. Confirmation by gas chromatography/mass spectrometry can be incorporated within this method with no additional sample preparation. A detection limit of 10 ng/mL was obtained by negative chemical ionization (–CI), with the base peak being the molecular ion. Using reduced solvent volumes or concentration of derivatized samples is necessary to detect low levels of the toxins.

A large percentage of T-2 and its metabolites in swine administered T-2 is found in the urine as glucuronide conjugates (Corley et al., 1985). These polar conjugates are not extracted with this method. Incubation of the urine with β-glucuronidase liberates the trichothecene metabolites (Corley et al., 1986). Analysis of the conjugates of trichothecenes can be accomplished with this method by incubating the sample with β-glucuronidase prior to extraction. With this screening method, no TOL or de-TOL was detected in the urine (collected at 1, 7, or 14 days postdosing) from pigs dosed dermally with T-2 at 15 mg/kg. After β-glucuronidase incubation of the urine (added directly to the C-18 cartridge as for nonincubated urine), de-TOL was detected at every time period. The levels ranged from 25 to 85 ng/mL total de-TOL.

Differences in C-18 cartridges from various manufacturers affected spike recoveries primarily for the more

polar trichothecenes such as NIV and TOL. With several brands of cartridges, the polar, hydrophilic toxins were not completely adsorbed onto the C-18 packing, although all of the cartridges efficiently extracted the more lipophilic toxins such as DAS and T-2. The recoveries of TOL and NIV from the various C-18 cartridges ranged from 10 to 98% and 95 to 99% for T-2. Inclusive calibration of the C-18 cartridges (i.e., with T-2 and TOL) is necessary to ensure reproducible and acceptable recoveries for both polar and nonpolar trichothecenes. Individual lots of the cartridges (C-18 and silica) should be checked since variation within lot numbers has been reported (Majors, 1986). Additionally, we have noted variation within individual lots of Florisil, which affected TOL recoveries in the same manner as for the C-18 cartridges. Volumes greater than 1.5 mL of urine or plasma, or highly colored samples, should be extracted with a 1-g C-18 cartridge rather than a 500-mg cartridge in order to prevent overloading of the C-18 packing with subsequent poor toxin extraction.

There are several advantages of this procedure over a method that analyzes for the parent compounds (T-2, DAS, DON, NIV) and/or individual metabolites. T-2 and DAS plasma levels drop rapidly following the administration of an iv dose. T-2 half-lives of 10–20 min in the blood of pigs (Beasley et al., 1986) and 5–10 min in the blood of dogs (Sintoz et al., 1986) have been reported. Many metabolites are present after the parent compounds can no longer be detected. This screening method can detect the presence of these metabolites at low levels in an easy and relatively rapid manner with confirmation by GC/MS. Trichothecene exposure can be determined long after the parent compound(s) can no longer be detected.

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